

REPORT DOCUMENTATION PAGE			Form Approved OMB NO. 0704-0188		
<p>The public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington VA, 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number.</p> <p>PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.</p>					
1. REPORT DATE (DD-MM-YYYY)		2. REPORT TYPE New Reprint		3. DATES COVERED (From - To) -	
4. TITLE AND SUBTITLE Conjugated oligoelectrolytes increase current response and organic contaminant removal in wastewater microbial fuel cells			5a. CONTRACT NUMBER		
			5b. GRANT NUMBER W911NF-09-D-0001		
			5c. PROGRAM ELEMENT NUMBER 611104		
6. AUTHORS Logan E. Garner, Alexander W. Thomas, James J. Sumner, Steven P. Harvey, Guillermo C. Bazan			5d. PROJECT NUMBER		
			5e. TASK NUMBER		
			5f. WORK UNIT NUMBER		
7. PERFORMING ORGANIZATION NAMES AND ADDRESSES University of California - Santa Barbara 3227 Cheadle Hall 3rd floor, MC 2050 Santa Barbara, CA 93106 -2050			8. PERFORMING ORGANIZATION REPORT NUMBER		
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Research Office P.O. Box 12211 Research Triangle Park, NC 27709-2211			10. SPONSOR/MONITOR'S ACRONYM(S) ARO		
			11. SPONSOR/MONITOR'S REPORT NUMBER(S) 55012-LS-ICB.521		
12. DISTRIBUTION AVAILABILITY STATEMENT Approved for public release; distribution is unlimited.					
13. SUPPLEMENTARY NOTES The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision, unless so designated by other documentation.					
14. ABSTRACT See attached.					
15. SUBJECT TERMS See attached.					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT UU	15. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON Francis Doyle
a. REPORT UU	b. ABSTRACT UU	c. THIS PAGE UU			19b. TELEPHONE NUMBER 805-893-8133

## **Report Title**

Conjugated oligoelectrolytes increase current response and organic contaminant removal in wastewater microbial fuel cells

## **ABSTRACT**

See attached.

---

**REPORT DOCUMENTATION PAGE (SF298)**  
**(Continuation Sheet)**

---

Continuation for Block 13

ARO Report Number    55012.521-LS-ICB  
Conjugated oligoelectrolytes increase current re    ...

Block 13: Supplementary Note

© 2012 . Published in Energy & Environmental Science, Vol. Ed. 0 5, (11) (2012), ( (11). DoD Components reserve a royalty-free, nonexclusive and irrevocable right to reproduce, publish, or otherwise use the work for Federal purposes, and to authroize others to do so (DODGARS §32.36). The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision, unless so designated by other documentation.

Approved for public release; distribution is unlimited.

## Conjugated oligoelectrolytes increase current response and organic contaminant removal in wastewater microbial fuel cells†

Logan E. Garner,<sup>a</sup> Alexander W. Thomas,<sup>a</sup> James J. Sumner,<sup>b</sup> Steven P. Harvey<sup>c</sup> and Guillermo C. Bazan<sup>\*a</sup>

Received 13th July 2012, Accepted 4th September 2012

DOI: 10.1039/c2ee22839d

The conjugated oligoelectrolyte 4,4'-bis(4'-(*N,N*-bis(6'-(*N,N,N*-trimethylammonium)hexyl)amino)-styryl)stilbene tetraiodide (DSSN<sup>+</sup>) has been employed to improve the performance of wastewater microbial fuel cells (MFCs) with respect to current generation and organic contaminant removal. The best performance was afforded by biocathode type MFCs run in the presence of DSSN<sup>+</sup>. Laser scanning confocal microscopy confirmed cellular uptake of DSSN<sup>+</sup> in the biofilms.

Conjugated oligoelectrolytes (COEs) contain a framework with a  $\pi$ -delocalized electronic structure and pendant groups with ionic functionalities. These molecules have been used in biosensors,<sup>1–5</sup> organic optoelectronic devices,<sup>6,7</sup> and in studies concerning the influence of polar media on linear and nonlinear photophysics of organic materials.<sup>8–13</sup> For example, COEs can be used to reduce the

charge injection barriers from electrodes into the semiconducting layer of polymer light emitting diodes.<sup>6</sup> Furthermore, electrostatic aggregates of cationic COEs and dye-labeled DNA possess optical and structural properties that allow discrimination of various proteins and bacteria.<sup>5</sup>

More recently, certain COEs have been demonstrated to spontaneously insert into liposomes and within the membranes of yeast.<sup>14</sup> The driving force for this spontaneous self-assembly stems from the hydrophobic aromatic core, which prefers the interior of the membrane so as to minimize contact with the aqueous environment. Moreover, there is evidence that membrane-spanning COEs improve the ability of electrons to transverse the lipid bilayer. A specific example concerns 4,4'-bis(4'-(*N,N*-bis(6'-(*N,N,N*-trimethylammonium)hexyl)amino)-styryl)stilbene tetraiodide (DSSN<sup>+</sup>). See Fig. 1 for the molecular structure of DSSN<sup>+</sup> and a cartoon illustration of its incorporation into a cell membrane. Introduction of yeast modified with DSSN<sup>+</sup> into microbial fuel cells (MFCs) leads to an increase of generated current, compared to unmodified yeast, which suggests improved interaction with the charge-collecting electrode.<sup>14</sup> In related work, membrane modification by way of Pd nanoparticles has led to improved charge transfer across insulating cell membranes to electrodes.<sup>15</sup>

Herein, we investigate to what extent DSSN<sup>+</sup> can induce electrogenic character, namely the ability to accept charge from or deliver charge to an electrode, in a diverse and not predetermined microbial population. From a broader scientific perspective, this problem concerns how to best interface internal metabolic pathways with an

<sup>a</sup>Department of Chemistry & Biochemistry, Department of Materials, Center for Polymers and Organic Solids, University of California, Santa Barbara, CA 93106, USA. E-mail: bazan@chem.ucsb.edu; Tel: +1 805 893-5538

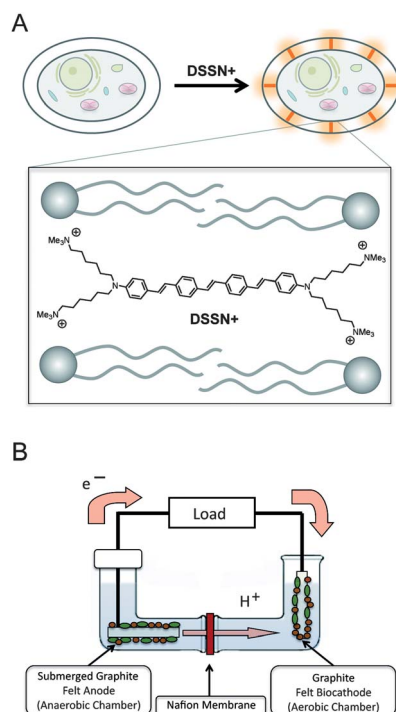
<sup>b</sup>Sensors and Electron Devices Directorate, U.S. Army Research Laboratory, Adelphi, MD 20783, USA. E-mail: james.j.sumner4.civ@mail.mil

<sup>c</sup>U.S. Army Edgewater Chemical Biological Center, 5183 Blackhawk Road, Building E3160, Aberdeen Proving Ground, MD 21010-5424, USA. E-mail: steve.harvey@us.army.mil

† Electronic supplementary information (ESI) available. See DOI: 10.1039/c2ee22839d

## Broader context

Bioelectrochemical processes are the basis of technologies that take advantage of microorganisms for generating electricity or carrying out chemical transformations. Throughout all types of device architectures one finds that the nature of the electrode–microbe interface is critical for charge extraction and/or injection. Not all microbes have developed electrogenic capabilities and there is great interest in finding suitable, and hopefully general, methods that can induce this behavior. Here, it is shown that conjugated oligoelectrolytes can be added to biocathode-type wastewater microbial fuel cells and thereby increase the conversion of carbon contaminants into electricity. Conjugated oligoelectrolytes are described by an electronically delocalized backbone and pendant ionic functionalities and their amphiphilic properties lead to spontaneous membrane intercalation. Of primary significance is that the microbial community in the wastewater was not predetermined. Thus, it appears that conjugated oligoelectrolytes can induce electrogenic behavior in a microbial system of undetermined composition. Such findings may lead to increases in conversion efficiency of wastewater into electricity, but from a broader perspective, they indicate the possibility of improving access to electroactive metabolic intermediates.



**Fig. 1** (A) The molecular structure of **DSSN+** and illustration of its incorporation into a cell membrane. The original counterions on **DSSN+** are iodide. (B) Illustration of the U-tube MFC design used in this study. Shown is a biocathode type MFC.

external circuit *via* specifically designed molecular materials. We find that, indeed, addition of **DSSN+** leads to better charge extraction from a microbial community present in wastewater onto carbon electrodes, and that these communities are more effective at consuming organic contaminants.

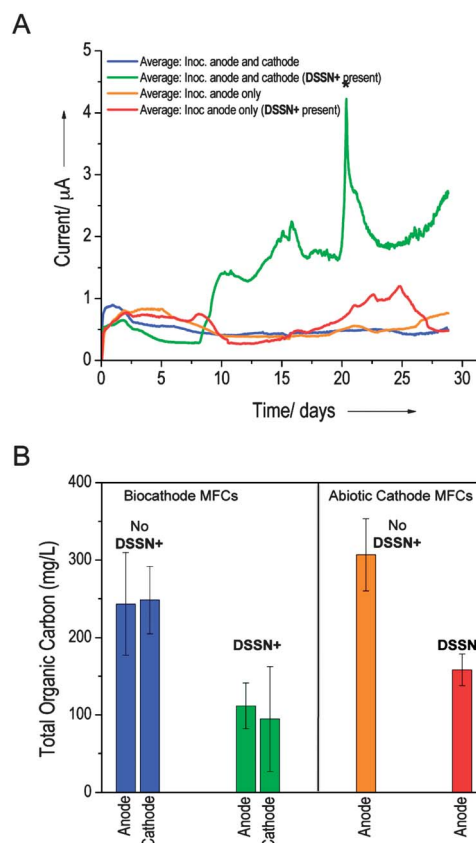
Wastewater MFCs were used as a testing platform.<sup>16–19</sup> Fig. 1B illustrates the MFC architecture used in this study.<sup>20,21</sup> In these devices, the microbial consortia that exist in a given waste stream oxidize organic contaminants in an anaerobic compartment containing an anode, generating electrons and protons. Electrons are donated to the anode and travel through an external circuit, while protons diffuse through a proton exchange membrane into an aerobic compartment containing the cathode, effectively balancing charge. Upon reaching the cathode, electrons then participate in reduction of oxygen, completing the overall MFC reaction.<sup>22,23</sup> MFCs employing microbial populations at the cathode as well as the anode have shown improved performance over abiotic cathode devices.<sup>24,25</sup> Changes in the generated current in MFC devices thus provide insight into the nature of the biofilm–electrode interfaces.

Two different MFC types were fabricated: (a) *abiotic cathode type* containing sterilized wastewater in the cathode chamber and (b) *biocathode type*, in which both the anode and the cathode chambers contain the as-received wastewater. The wastewater employed in our studies comprised of the spent medium from the mixed culture anoxic dark fermentation of paper.<sup>26</sup> For each MFC type, half of the runs were carried out in the absence and half in the presence of 5  $\mu$ M **DSSN+**. No additional nutrients, organisms or organic compounds were added to the waste stream.

Results from operating MFCs plotted as a function of current *versus* operation time are shown in Fig. 2A (results from a duplicate

set have also been plotted to demonstrate reproducibility and can be found in Fig. S1, ESI†). Current values were calculated based on the potential measured across a 10 k $\Omega$  resistor. MFCs run in the absence of **DSSN+** (blue and orange curves) produce little current over the course of 30 days. Abiotic cathode MFCs run in the presence of **DSSN+** (red curve) begin to produce notable current after 20 days and reach maxima in the  $\sim 1.5$  to 3  $\mu$ A range. Biocathode MFCs run with **DSSN+** (green curve) exhibit the best performance, producing current after 8 days and reaching maxima in the range of  $\sim 3.5$  to 4.5  $\mu$ A. Most importantly the collected set of data demonstrates a substantial increase in the current generated upon introduction of **DSSN+**. Furthermore, the impact is most pronounced when the as-received wastewater, and by inference a population of microorganisms, is present in both the anode and cathode chambers. That addition of **DSSN+** results in the most pronounced performance increase for biocathode type MFCs suggests that the COE influences microbe–electrode interactions at both anode and cathode.

Removal of organic contaminants was quantified by examination of the total organic carbon (TOC) and the chemical oxygen demand (COD) of the MFC effluent after operation. TOC is measured based



**Fig. 2** (A) Performance of wastewater MFCs. Those run in the absence of **DSSN+** (blue and orange) yield little current, while those employing **DSSN+** (green and red) yield  $\sim 2$  to 8 times greater current. Each trace represents the average of duplicate MFC sets. \* indicates a spike in current that occurred upon dislodging gas bubbles formed during operation. (B) Comparison of the total organic carbon remaining in MFC effluent after  $\sim 30$  days of operation. MFCs were run in the absence (blue and orange) and presence (green and red) of 5  $\mu$ M **DSSN+**. For biocathode type MFCs the effluent from both chambers was analyzed while the effluent from only the anode chambers was analyzed for abiotic cathode type MFCs. Note:  $\text{TOC}_{\text{initial}} = 970 \text{ mg L}^{-1}$ .

on the CO<sub>2</sub> released by oxidation of contaminants, whereas COD quantifies the amount (in mg L<sup>-1</sup>) of O<sub>2</sub> required to oxidize the contaminants, and can be directly compared to current production.<sup>27</sup> Fig. 2B shows the results from the TOC measurements (reported as an average of three datasets). For biocathode MFCs, one observes that the anode and cathode chambers contain similar levels of contaminants. However, MFCs to which **DSSN+** was added show lower remaining TOC values, ~100 mg L<sup>-1</sup> vs. ~250 mg L<sup>-1</sup>. Abiotic cathode type MFCs exhibit a similar trend in terms of remaining TOC between runs in the presence and absence of **DSSN+**. Microbial populations functioning in the presence of **DSSN+** are therefore more effective at metabolizing the organic contaminants in the wastewater. Moreover, the fact that the contaminant concentration remaining in the biocathode MFCs is lower relative to their abiotic cathode counterparts suggests complementary metabolic processes between microbial populations in the anode and cathode chambers.

The COD remaining after MFC operation for each set of conditions (Fig. S2, ESI†) followed the same trend observed in TOC analysis: 488 mg L<sup>-1</sup> vs. 858 mg L<sup>-1</sup> for biocathode type MFCs run with and without **DSSN+**, respectively, and 1037 mg L<sup>-1</sup> vs. 1322 mg L<sup>-1</sup> for abiotic cathode type MFCs run with and without **DSSN+**, respectively. Assuming 4 moles of available electrons per mole COD,<sup>28</sup> these differences correspond to the additional charge

available for collection of 89 coulombs (370 mg L<sup>-1</sup> COD) for biocathode type and 69 coulombs (285 mg L<sup>-1</sup> COD) for abiotic cathode type. However, the additional charge actually harvested by MFCs run with **DSSN+** was ~2.3 coulombs and ~0.5 coulombs for biocathode and abiotic cathode MFCs, respectively, obtained by integrating the current vs. time plots in Fig. 2A and S1 (ESI†). Thus, while the increase in current generation afforded by **DSSN+** is significant, it only accounts for a small portion of the increased COD consumption.

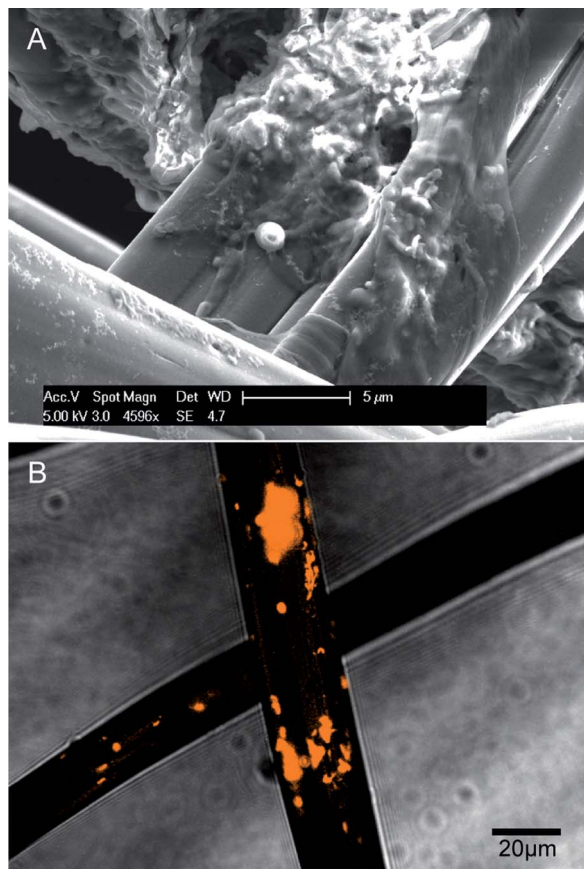
Fig. 3A shows a scanning electron micrograph (SEM) of a carbon felt anode after the operation of a biocathode type MFC run in the presence of **DSSN+**. Accumulation of biomass originating from the microbial population initially present in the wastewater is observed on the electrode surface. It should be noted that the biomass features range in shape, morphology and density throughout the electrode fibers (Fig. S4, ESI†), consistent with biofilm growth from a consortium of microorganisms. It is clear, however, that the coverage is not complete as would be expected from the fact that no effort was made to specifically grow a biofilm on the electrode surface. Fig. 3B displays a fluorescence image of the carbon felt fibers obtained *via* laser scanning confocal microscopy overlaid atop the corresponding bright field image. The emission was obtained by excitation of **DSSN+** *via* a 488 nm laser excitation source. Since no other fluorophore was added in these experiments, we surmise that the emission originates from **DSSN+**. The similarity between the images in Fig. 3 suggests that **DSSN+** has accumulated within the microorganism colonies. Furthermore, an emission spectrum corresponding to emissive regions of a confocal microscopy image (405 nm excitation) of an electrode surface (Fig. S3, ESI†) showed a maximum intensity at ~485 nm, consistent with the emission profile of **DSSN+** in a non-polar environment.<sup>14</sup> This observation suggests that **DSSN+** is likely incorporated into the lipid membranes of the microbes comprising the biofilms.

## Conclusions

In conclusion we have demonstrated that the simple addition of dilute solutions containing the synthetic conjugated oligoelectrolyte **DSSN+** enhances the ability of an undetermined microbial community to interact with electrodes. The microbial community also more efficiently metabolizes organic contaminants. These improvements were most pronounced when **DSSN+** was introduced into biocathode type MFCs. The biomass on the electrode surfaces contained **DSSN+**, most likely as a result of membrane intercalation, although whether the COE must exist in cell membranes to facilitate microorganism–electrode interactions is still unclear and under investigation. It is worth noting, however, that **DSSN+** is unlikely to act as a traditional, diffusive redox mediator, as it does not undergo reduction or oxidation within the potential window of an operating MFC.<sup>14</sup> An important consequence is that this study demonstrates the ability to induce electrogenic characteristics in a microbial system of undetermined composition. That **DSSN+** introduction into both anodic and bio-cathodic chambers leads to the largest current generation and TOC consumption suggests a synergistic effect on both injection and extraction of charges.

## Acknowledgements

The authors thank the Institute for Collaborative Biotechnologies (W911NF-09-D-0001), and the Air Force Office of Scientific



**Fig. 3** (A) SEM and (B) overlaid bright field and confocal images of the carbon felt fibers (dark structures) of the anode of a biocathode type MFC run in the presence of **DSSN+**. No additional stain was added prior to imaging. The emission is thus attributed to **DSSN+** accumulated within the electrode biomass.



Research (FA9550-08-1-0248) for financial support; the National Center for Research Resources Shared Instrument Grant (1S10RR017753-01) for funding of confocal microscopy facilities employed in this work; the National Science Foundation (DMR05-20415) for support of Materials Research Laboratory (MRL) central facilities that house the SEM instrument employed in this work; Mr Mark Cornish for assistance in SEM imaging; and Dr Christian J. Sund and Dr Amethyst S. Finch for technical assistance and consultation in the operation of wastewater MFCs.

## Notes and references

- 1 Y. Chen, H. Bai, Q. Chen, C. Li and G. Shi, *Sens. Actuators, B*, 2009, **138**, 563–571.
- 2 A. Herland, P. R. Nilsson, J. D. M. Olsson, P. Hammarstrom, P. Konradsson and O. Inganas, *J. Am. Chem. Soc.*, 2005, **127**, 2317–2323.
- 3 J. W. Hong, H. Benmansour and G. C. Bazan, *Chem.–Eur. J.*, 2003, **9**, 3186–3192.
- 4 K. Y. Pu, K. Li, X. Zhang and B. Liu, *Adv. Mater.*, 2010, **22**, 4186–4189.
- 5 H. Li and G. C. Bazan, *Adv. Mater.*, 2009, **21**, 964–967.
- 6 R. Yang, Y. Xu, X.-D. Dang, T.-Q. Nguyen, Y. Cao and G. C. Bazan, *J. Am. Chem. Soc.*, 2008, **130**, 3282–3283.
- 7 Y. Xu, R. Yang, J. Peng, A. A. Mikhailovsky, Y. Cao, T.-Q. Nguyen and G. C. Bazan, *Adv. Mater.*, 2009, **21**, 584–588.
- 8 C. R. Chenthamarakshan and A. Ajayaghosh, *Chem. Mater.*, 1998, **10**, 1657–1663.
- 9 B. S. Gaylord, S. Wang, A. J. Heeger and G. C. Bazan, *J. Am. Chem. Soc.*, 2001, **123**, 6417–6418.
- 10 M. Stork, B. S. Gaylord, A. J. Heeger and G. C. Bazan, *Adv. Mater.*, 2002, **14**, 361–366.
- 11 G. Zotti, S. Zecchin, G. Schiavon and L. B. Groenendaal, *Macromol. Chem. Phys.*, 2002, **203**, 1958–1964.
- 12 E. Collini, C. Ferrante and R. Bozio, *J. Phys. Chem. B*, 2005, **109**, 2–5.
- 13 H. Y. Woo, B. Liu, B. Kohler, D. Korystov, A. Mikhailovsky and G. C. Bazan, *J. Am. Chem. Soc.*, 2005, **127**, 14721–14729.
- 14 L. E. Garner, J. Park, S. M. Dyar, A. Chworos, J. J. Sumner and G. C. Bazan, *J. Am. Chem. Soc.*, 2010, **132**, 10042–10052.
- 15 X. Wu, F. Zhao, N. Rahunen, J. R. Varcoe, C. Avignone-Rossa, A. E. Thumser and R. C. T. Slade, *Angew. Chem., Int. Ed.*, 2011, **50**, 427–430.
- 16 J. Cha, S. Choi, H. Yu, H. Kim and C. Kim, *Bioelectrochemistry*, 2010, **78**, 72–79.
- 17 Y. Feng, X. Wang, B. E. Logan and H. Lee, *Appl. Microbiol. Biotechnol.*, 2008, **78**, 873–880.
- 18 F. Kargi and S. Eker, *J. Chem. Technol. Biotechnol.*, 2009, **84**, 961–965.
- 19 T. Shimoyama, S. Komukai, A. Yamazawa, Y. Ueno, B. E. Logan and K. Watanabe, *Appl. Microbiol. Biotechnol.*, 2008, **80**, 325–330.
- 20 C. E. Milliken and H. D. May, *Appl. Microbiol. Biotechnol.*, 2006, **73**, 1180–1189.
- 21 C. J. Sund, M. S. Wong and J. J. Sumner, *Biosens. Bioelectron.*, 2009, **24**, 3144–3147.
- 22 D. R. Lovley, *Curr. Opin. Biotechnol.*, 2008, **19**, 564–571.
- 23 F. Zhao, R. C. Slade and J. R. Varcoe, *Chem. Soc. Rev.*, 2009, **38**, 1926–1939.
- 24 S. V. Mohon and S. Srikanth, *Bioresour. Technol.*, 2011, **102**, 10210–10220.
- 25 Z. He and L. T. Angenent, *Electroanalysis*, 2006, **18**, 2009–2015.
- 26 S. Harvey and M. Dixon, *Int. J. Hydrogen Energy*, 2010, **35**, 9611–9617.
- 27 C. P. L. Grady Jr, G. T. Daigger and H. C. Lim, *Biological Wastewater Treatment*, CRC Press, New York, 1999.
- 28 H. Liu, R. Ramnarayanan and B. E. Logan, *Environ. Sci. Technol.*, 2004, **38**, 2281–2285.